dichotomous effect described and to achieve high levels of both packaging and translation, it is also desirable to position AUG and/or termination codons within the intron of the vector. A sample set of oligonucleotides is illustrated below for creating a region which has the following structural features: Cla1 compatible ends for insertion into VLCN; multiple advantageous ATG codons, followed quickly by termination codons for abortive translation; a splice acceptor site homologous to the AKV virus splice acceptor site (to give partial, but not complete splicing in cells); and several unique and useful restriction endonuclease sites.

ATGSACU UPPER STRAND, CLA1 OVERHANGS WHEN [ANNEALLED] ANNEALED, NO CLA1 SITE 5'-
CGGAAATGATCATGGAATGATAAGATGACCTAACTAATAGCCCATCTCTCCAAGATC
GATCAGGCCTAGATCT-3' (SEQ ID NO:15)

ATGSACB BOTTOM STRAND
5'-
CGAGATCTAGGCCTGATCGATCTTGGAGAGATGGGCTATTAGTTAGGTCATCTTATC
ATTCCATGATCATTTC-3' (SEQ ID NO:16)

Remarks

This Supplemental Preliminary Amendment and the above-referenced SEQUENCE LISTING are filed to conform the above-referenced application to the requirements of 37 C.F.R. §§ 1.821 - 1.825. To conform the above-referenced application to the requirements of 37 C.F.R. §§ 1.821 through 1.825, a paper copy of a Sequence Listing is submitted herewith. The paper copy of the Sequence Listing in this application is identical to the computer readable form of the Sequence Listing filed in application Serial No. 08/522,336, filed November 09, 1995. In accordance with 37 C.F.R. § 1.821(e), please use the computer readable form filed on October 14, 1998 in application Serial No. 08/522,336 as the computer readable form for the instant application.

The amendments to the specification, to add sequence identifiers, are made to conform the above-referenced application to the requirements of 37 C.F.R. § 1.821(d). Amendments were also made to the specification to correct typographical errors. It is respectfully submitted that these amendments do not represent new matter.

Respectfully submitted,

CLAGUE P. HODGSON,

By his Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6959

Date November 13, 2001 By _____
Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 13th day of November, 2001.

Candis B. Buending
Name

_____
Signature

CLEAN VERSIONS OF SUBSTITUTE PARAGRAPHS FOR SPECIFICATION

The section on page 1 titled RELATED APPLICATIONS, added by Preliminary Amendment dated August 21, 2001, as follows:

RELATED APPLICATIONS

This is a Continuation Under 1.53(b) of U.S. Application Serial No. 08/522,336, filed November 9, 1995, which is a U.S. National Stage filing under 35 U.S.C. 371 of PCT/US94/02752, filed March 14, 1994 (published as WO 94/20608 on September 15, 1994), and which is a continuation-in-part of Application No. 08/194,208, filed on February 7, 1994, now abandoned, which is a continuation-in-part of application No. 08/130,638, filed on October 1, 1993, now abandoned, which is a continuation-in-part of application No. 08/097,721, filed on July 26, 1993, now abandoned, which is a continuation-in-part of application No. 08/060,568, filed on May 12, 1993, now abandoned, which is a continuation-in-part of application No. 08/030,766, filed on March 12, 1993, which applications are incorporated herein by reference.

The paragraph beginning on page 27, line 12 is amended as follows:

B. Primers and amplification reactions.

Oligonucleotide primers were made by Genosys Biotechnologies, Inc., Houston, Texas. Gene amplification reactions were performed in 100 µl of 10 mM Tris.HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM of deoxyribonucleoside triphosphate, 2.5 units of *taq* DNA polymerase (from *Thermus aquaticus*), 10 ng plasmid pNVLOVHGH (containing the complete NVL3 genome) and 1 µg of each primer. [Note: any suitable VL30 template, such as one of the many cloned VL30 DNA sequences, or mouse genomic DNA, can be used as a template]. Reactions proceeded through 35 cycles of denaturation (94°C for 1 min), primer annealing (56°C for 2 min), and primer extension (72°C for 3 min). In most cases the annealing temperature was 5°C below the calculated denaturing temperature. Sequences of the primers were as follows (5"-3"):

P1-5'-TCAGCAGATCTTGAAGAATAAAAAATTACTGGCCTCTTG-3' (SEQ ID NO:1),

P2-5'-AAGGGCGGCCGCTTAATTAATCCCTGATCCTCCCCTGTTCCTC-3' (SEQ ID NO:2),

P5-5'-ACTGCGGCCGCATAGACTTCTGAAATTCTAAGATTA-3' (SEQ ID NO:3),

P6 5'-GAAGATCTTGAAAGATTTTCGAATTCCCGGCCAATGC-3' (SEQ ID NO:4),

P7 5'-AAGGCGGCCGCTTAATTAATCTAAGGCCGGCCAATTGAGACC-3' (SEQ ID NO:5),

N5 5'-GGTTAATTAATTAGATCTAGCATGATTGAACAAGATGGATTGCAC-3' (SEQ ID NO:6),

N3 5'-TACTTAATTAACCATGGATCCGTTAACTCCGAAGCCCAACCTTTCATAG-3' (SEQ ID NO:7),

N3S 5'-
[']TACTTAATTAACCATGGTCTAGTGGATCCGACCTTGGAGAGAGAGAGTCAGTGTTA
ACTCCGAAGCCCAACCTTTCATAG-3' (SEQ ID NO:8).

The paragraph beginning at page 27, line 3 and continuing to page 38 line 7, is amended as follows:

4. Additional primers made for LTR substitutions

BGL2RU5 5'-TTTAGATCTTCCCTCCCCATTCCCCCTCCCAGTT-3' (SEQ ID NO:9)

3PHETLTR 5'-CGAGGTACCTGAAAGA(CT)(CT)(CT)(CT)CG-3' (SEQ ID NO:10)

MCSP3P 5'-GGGTTCAGATCTTGATCAG-3' (SEQ ID NO:11)

3LTR5MCS 5'-
TAAGCGGCCGCTAGACTTCTGAAATTCTAAGATTAGAATTATTTACAAGAAGAAGTG-
GGGAATGAAGAATAAAAAATTCTGATCAAGATCTGAACCC-3' (SEQ ID NO:12)

3LTR5 5'-
TAAGCGGCCGCTAGACTTCTGAAATTCTAAGATTAGAATTATTTACAAGAAGAAGTG
GGGAATGAA-3' (SEQ ID NO:13)

KPN1IRU5 5'-CGAGGTACCTGAAAGATTTTCGAATTCCCGGCCAAT-3' (SEQ ID NO:14)

The paragraph beginning at page 41, line 20 and continuing to page 42 line 22, is amended as follows:

For gene therapy, it would be especially desirable to have a vector which has both high titer as well as strong protein expression. This can be attained by combining AUG start codons with splicing of the 5'-leader sequence. Unspliced vectors are packaged efficiently because translation is frequently aborted. In the recipient cell, processing of a 5'-intron containing AUG codons and packaging signals permit more efficient translation of a protein product, especially if it resulted in the removal of confounding ATG codons. Thus, it would be desirable to have a

splice donor and acceptor site in the 5'-end of the RNA which would permit some percentage (less than 100%) of the RNA molecules to be spliced. Ideally, it would be desirable to have efficient splicing in the recipient cell, but not in the producer (donor, or helper) cell. The synthetic vectors shown in FIG. 2 have splice donor site [concensus] consensus sequences just [preceeding] preceding the packaging signal. It is possible to insert a splice acceptor sequence into a unique restriction endonuclease site, such as the *Cla1* site of VLCN or its derivatives, or the *Dra3* site of VLDN. However, in order for this to have greater effect, it is also desirable to mutagenize some or all of the confounding AUG condons which lie outside the splice region. This can be done by using any techniques of site-directed mutagenesis (Ausubel, *supra*; or, for example, using the commercially available kit with manufacturers instructions, Stratagene #200510, LaJolla, CA; ref: Felts, K., *et al.* 1992, Strategies 5:26-28). Alternatively, it is possible to use a splice donor which is farther upstream, for example, in the LTR. To enhance the dichotomous effect described and to achieve high levels of both packaging and translation, it is also desirable to position AUG and/or termination codons within the intron of the vector. A sample set of oligonucleotides is illustrated below for creating a region which has the following structural features: Cla1 compatible ends for insertion into VLCN; multiple advantageous ATG codons, followed quickly by termination codons for abortive translation; a splice acceptor site homologous to the AKV virus splice acceptor site (to give partial, but not complete splicing in cells); and several unique and useful restriction endonuclease sites.

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ATGSACB BOTTOM STRAND

5'-

CGAGATCTAGGCCTGATCGATCTTGGAGAGATGGGCTATTAGTTAGGTCATCTTATC ATTCCATGATCATTTC-3' (SEQ ID NO:16)